

Integrative data reveal a new millipede species of *Sinocallipus* Zhang, 1993 (Callipodida, Sinocallipodidae) from Vietnam, with notes on its phylogeny

Anh D. Nguyen^{1,2}, Pavel Stoev^{3,4}, Tam T. T. Vu^{1,2}

¹ Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, 18, Hoangquocviet Rd., Cau Giay District, Hanoi, Vietnam

² Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18, Hoangquocviet Rd., Cau Giay District, Hanoi, Vietnam

³ National Museum of Natural History, Bulgarian Academy of Sciences, Tsar Osvoboditel Blvd. 1, 1000 Sofia, Bulgaria

⁴ Pensoft Publishers, Prof. G. Zlatarski Str. 12, Sofia, Bulgaria

<https://zoobank.org/0F9E72F6-2E43-4B0B-9BAD-29F2EF728396>

Corresponding author: Anh D. Nguyen (ducanh.iebr@gmail.com, ndanh@iebr.vast.vn)

Academic editor: Luiz F. Iniesta ♦ Received 8 October 2024 ♦ Accepted 22 November 2024 ♦ Published 6 January 2025

Abstract

The callipodidan genus *Sinocallipus* Zhang, 1993 (Callipodida, Sinocallipodidae) is reviewed within the scope of the Vietnamese fauna. A total of three species are recorded in Vietnam including a new one, *Sinocallipus similis* sp. nov. All three species are confirmed by morphological and molecular data. An existing identification key of *Sinocallipus* species is amended to include the new species.

Key Words

Biodiversity, cave fauna, COI, phylogeny, taxonomy, Vietnam

Introduction

The family Sinocallipodidae Zhang, 1993 was established for the monotypic genus *Sinocallipus* Zhang, 1993 (with *Sinocallipus simplipodicus* Zhang, 1993) from Xiao Cave, Hekou Yaozu Autonomous County, Yunnan Province, southern China (Zhang 1993). The family is considered to be the first group to diverge of all extant callipodidans and is placed in its own suborder, Sinocallipodidea (Shear 2000; Shear et al. 2003). Unlike other callipodidans, which are primarily found in temperate zones of the Northern Hemisphere, representatives of the genus *Sinocallipus* inhabit the subtropical and tropical zones of Southeast Asia, between approximately 14°N and 22°N.

In addition to the type species from China, five species have been described from Southeast Asia in the last 17 years: *Sinocallipus thai* Stoev et al., 2007 from the surroundings of Sri Wilai Temple, Saraburi Province, Thai-

land; *Sinocallipus deharvengi* Stoev & Enghoff, 2011 from caves in Quang Binh Province, central Vietnam; *Sinocallipus catba* Stoev & Enghoff, 2011 from Hoa Cuong Cave and Tien Duc Cave, Cat Ba National Park, Hai Phong Province, northern Vietnam; *Sinocallipus jaegeri* Stoev & Enghoff, 2011 from caves in Khammouan Province, Laos; and *Sinocallipus steineri* Stoev & Enghoff, 2011 from caves in Luang Phrabang Province, Laos (Stoev et al. 2007; Stoev and Enghoff 2011). Thus, the genus currently comprises 6 formally described species (see Stoev and Enghoff 2011 for more details).

Other *Sinocallipus* specimens of unclear taxonomic identity have been reported from Vietnam: Hanoi City (Enghoff et al. 2004), Lang Son Province, Huu Lung Area, Snake Cave, and a cave 97 km north of Hanoi (Stoev and Enghoff 2011). Additional reports from Laos include specimens from Champasak and Attapu Provinces, Dong Hua Sao National Biodiversity Conservation Area,

along the Houry Phak River near the southwest edge of the Bolavens Plateau, at 15°04'37"N, 106°10'45"E (see Shear et al. 2003).

In the present paper, we review the genus *Sinocallipus* in Vietnam, describe one new species from caves in Tuyen Quang Province and report new data on the two species previously identified from the country.

Materials and methods

The millipede specimens treated in the present paper were collected from caves in Vietnam and preserved in 90% ethanol. All morphological terminology follows Stoev and Enghoff (2011). The holotype and most paratypes of the new species described are deposited in the Myriapod collection of the Institute of Ecology and Biological Resources (IEBR-Myr), Hanoi, Vietnam. Some paratypes are deposited in the National Museum of Natural History (NMNHS), Sofia, Bulgaria.

Morphological characters were examined using an Olympus SZX10 stereomicroscope. Gonopods were dissected for detailed morphological analysis and photographed. Color images were taken at different focal planes using a Sony a6000 camera attached to a SMZ800N Nikon stereomicroscope. Ultraviolet (=UV) images, which were better to show biofluorescent characters, were taken with the same camera setup under a Nichia Convoy UV flashlight. The images were stacked using Helicon Focus version 7.0 and further processed in Adobe Photoshop CS6.

DNA was extracted using Qiagen DNeasy Blood and Tissue Kits. A 680-bp fragment of the mitochondrial gene, cytochrome c oxidase subunit I (COI), was amplified and sequenced using a pair of universal primers, LCO1490 and HCO2198 (Folmer et al. 1994). Polymerase chain reaction (PCR) conditions for amplification of the COI gene follow those of Nguyen et al. (2017). The successfully amplified PCR products were sent to the GenLab company (Vietnam) for purification and sequencing. COI sequences were verified using BLASTN 2.6.0+ (Zhang et al. 2000) and registered in GenBank with unique accession numbers. All verified sequences were aligned using multiple sequence alignment with the program ClustalX ver. 2 (Larkin et al. 2007). Genetic distances between callipodidan samples were calculated using the Kimura 2-parameter model in MEGA ver.7.0 (Kumar et al. 2016). A maximum likelihood bootstrap analysis was conducted using the IQTREE server with 1,000 replicates at <http://iqtree.cibiv.univie.ac.at/> (Trifinopoulos et al. 2016).

Results

Taxonomy

Order Callipodida

Family Sinocallipodidae Zhang, 1993

Genus *Sinocallipus* Zhang, 1993

Figs 1A–D, 10

Sinocallipus Zhang, 1993: 129. Type species: *Sinocallipus simplipodius* Zhang, 1993, by original designation.

Sinocallipus catba Stoev & Enghoff, 2011.

Sinocallipus catba: Stoev & Enghoff, 2011: 16.

Material examined. VIETNAM • 2 ♀♀; Hai Phong Province, Cat Ba National Park, Trung Trang Cave; 20.7887°N, 106.9980°E; May 2012; Anh D Nguyen leg.; IEBR-Myr 542.

Diagnosis. Although only female specimens were collected and examined in the present study, the identity of the species is clear and well-supported. *S. catba* can be distinguished from its congeners by its white-yellowish body (Fig. 1A), a black eye composed of more than 30 ommatidia (Fig. 1B), and long antennae that extend beyond the posterior edge of pleurotergite (=PT) 7 when folded backwards. It also has 5+5 crests between the ozopores on midbody pleurotergites (=PTs), a gonocoxal process *g* that is approximately $1.5 \times$ the length of process *k*, and paraprocts divided into larger ventral and smaller dorsal sclerites (Fig. 1C; Stoev and Enghoff 2011). The females studied here have well-developed, highly elevated cylindrical cyphopods (Fig. 1D).

Remarks. The species is currently known from three caves on Cat Ba Island: Hoa Cuong Cave and Tien Duc Cave (Stoev and Enghoff 2011), and Trung Trang (present study).

Sinocallipus deharvengi Stoev & Enghoff, 2011

Figs 2A–D, 10

Material examined. VIETNAM • 2 ♂♂, 1 ♀; Quang Binh Province, Bo Trach District, Tu Lan Cave; 17.7729°N, 106.0901°E, 16–21 January 2023; TV Le leg.; IEBR-Myr 962 • 3 ♂♂, 2 ♀♀; Quang Binh Province, Bo Trach District, Tu Lan Cave; 17.7729°N, 106.0901°E; 16–21 January 2023; TV Le leg.; IEBR-Myr 1015.

Diagnosis. The species is morphologically similar to *Sinocallipus catba* from which it can be distinguished by its larger body, a gonocoxal process *g* that is more than $3 \times$ the length of process *k*, a differently shaped trochanteral process of leg 9 in males, and paraprocts divided into two, nearly equal-sized sclerites (Stoev and Enghoff 2011).

Remarks. The species was described originally from three caves situated in the region of Phong Nha and Cha Noi communes in Quang Binh Province. It is now reported from another cave (Tu Lan), located approximately 15 km northeast from the other localities at Cha Noi.

Sinocallipus similis sp. nov.

<https://zoobank.org/EE4CCF75-6BA6-417E-B839-98C623EFD758>

Figs 3–8, 9A, 10

Material examined. Holotype. VIETNAM • ♂; Tuyen Quang Province, Lam Binh District, Na Hang Nature

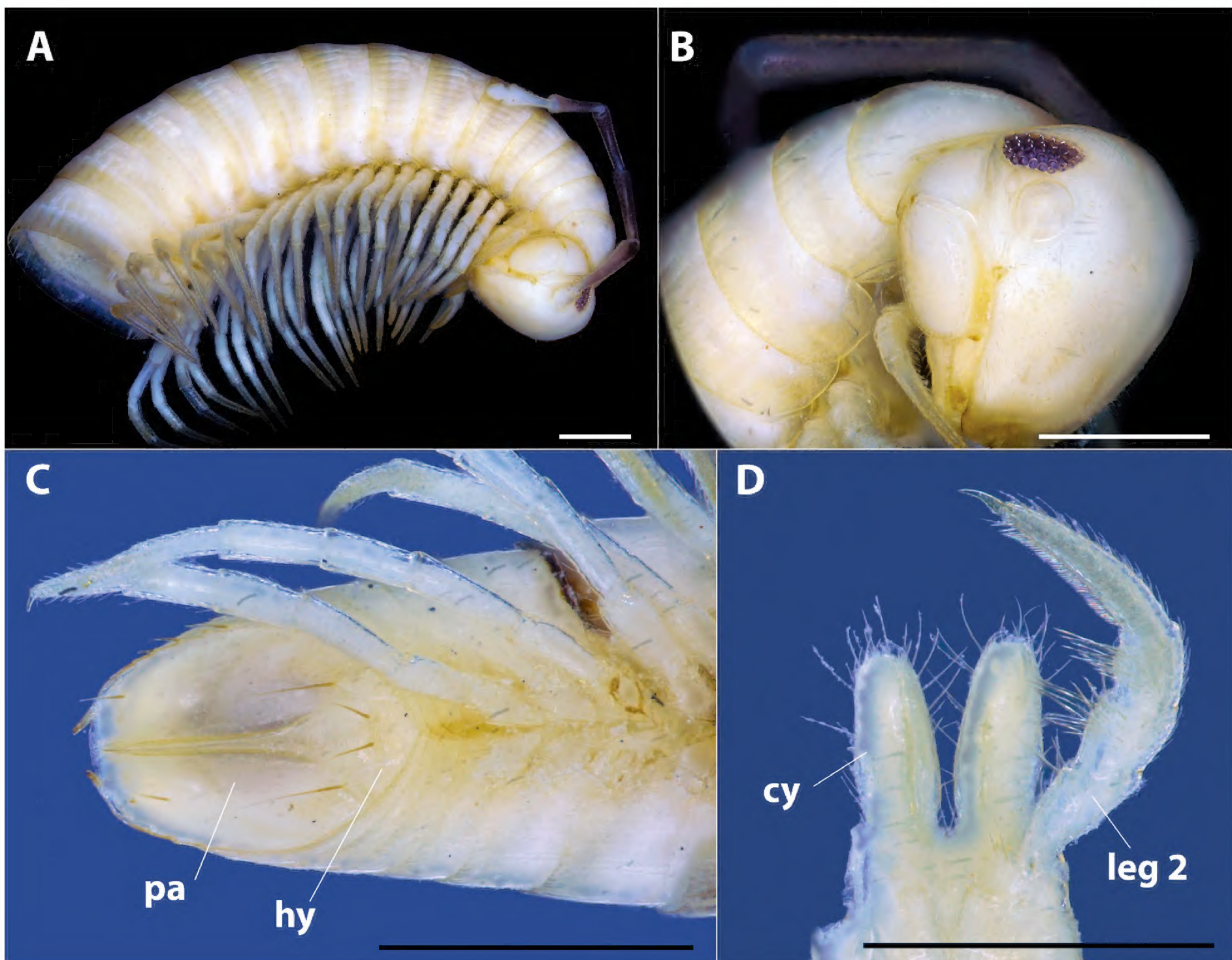


Figure 1. *Sinocallipus catba* Stoev & Enghoff, 2011. ♀, IEBR-Myr 542 **A.** Anterior part of body, lateral view; **B.** Head and ocelli, lateral view; **C.** Telson, ventral view; **D.** Cyphopods and leg 2, anterior view. Abbreviations: pa = paraprocts; hy = hypoproct; cy = cyphopods. Scale bars: 1 mm.

Reserve, Khuoi Pin Cave; 22.57134°N, 105.30977°E; 457 m a.s.l.; 26 June 2024; XS Le leg.; IEBR-Myr 994H.

Paratypes. VIETNAM • 2♂♂, 1♀; same as for holotype; IEBR-Myr 994P • 3♂♂, 1♀; same locality as the holotype, but Na Mang Cave, 22.45405°N, 105.32204°E, 305 m a.s.l.; 25 June 2024; XS Le leg.; IEBR-Myr 990 • 1♂, 1♀; same data as for IEBR-Myr 990; NMNHS • 1♀, 2 juvs.; same data as for IEBR-Myr 990; IEBR-Myr 991.

Description of type locality. The species was discovered in two caves within the Na Hang Nature Reserve, Tuyen Quang Province in northern Vietnam. Khuoi Pin Cave is located on the mountainside, measuring approximately 300 meters in length and 10 meters in height. The cave floor consists of sandy soil and small rocks. It is notably wet, with some water flow on the ground, and the air humidity is around 90%, while the temperature remains consistently at 20 °C. The cave also contains numerous small branches contributing to its unique ecosystem (Fig. 3A, C, D).

Na Mang Cave is situated near the base of the mountain. Its floor consists of sandy soil. Similar to Khuoi Pin Cave, it exhibits high humidity levels (around 90%) and maintains a consistent temperature of 20 °C. The cave is small and narrow with water flows on the ground (Fig. 3B).

Both caves are recognized for their rich biodiversity, hosting a variety of species, including several unidentified glyphiulid, haplodesmid and callipodidan millipedes, as well as spiders, crickets, and bats. Notably, all millipede specimens were gathered from the aphotic zone in both caves, indicating that the species are specialized to the cave environment.

Etymology. From the Latin word “*similis*” meaning “*similar*” or “*like*”. The name denotes the morphological similarity between the new species and *Sinocallipus deharvengi* from Quang Binh Province in Vietnam.

Diagnosis. This species can be recognized by having up to 79 pleurotergites (in adult females, males with 78), yellowish body, long antennae, 5+5 crests between the ozopores on midbody PTs, almost equally subdivided paraprocts, gonopods with strongly swollen and long gonocoxal process *g*, and a long, trochanteral process of leg 9 with a pointed tip.

Diagnosis remarks. It can be distinguished from *S. deharvengi* by antennal length (9.5 mm in *S. similis* vs. 9.7 mm in *S. deharvengi*), antennal coloration (antennomere 2 yellow, antennomeres, 3–5 partially brown in *S. similis* vs. antennomere 2–5 brown in *S. deharvengi*), different shape of trochanteral process *h* (long with a pointed tip in *S. similis* vs. short in *S. deharvengi*)

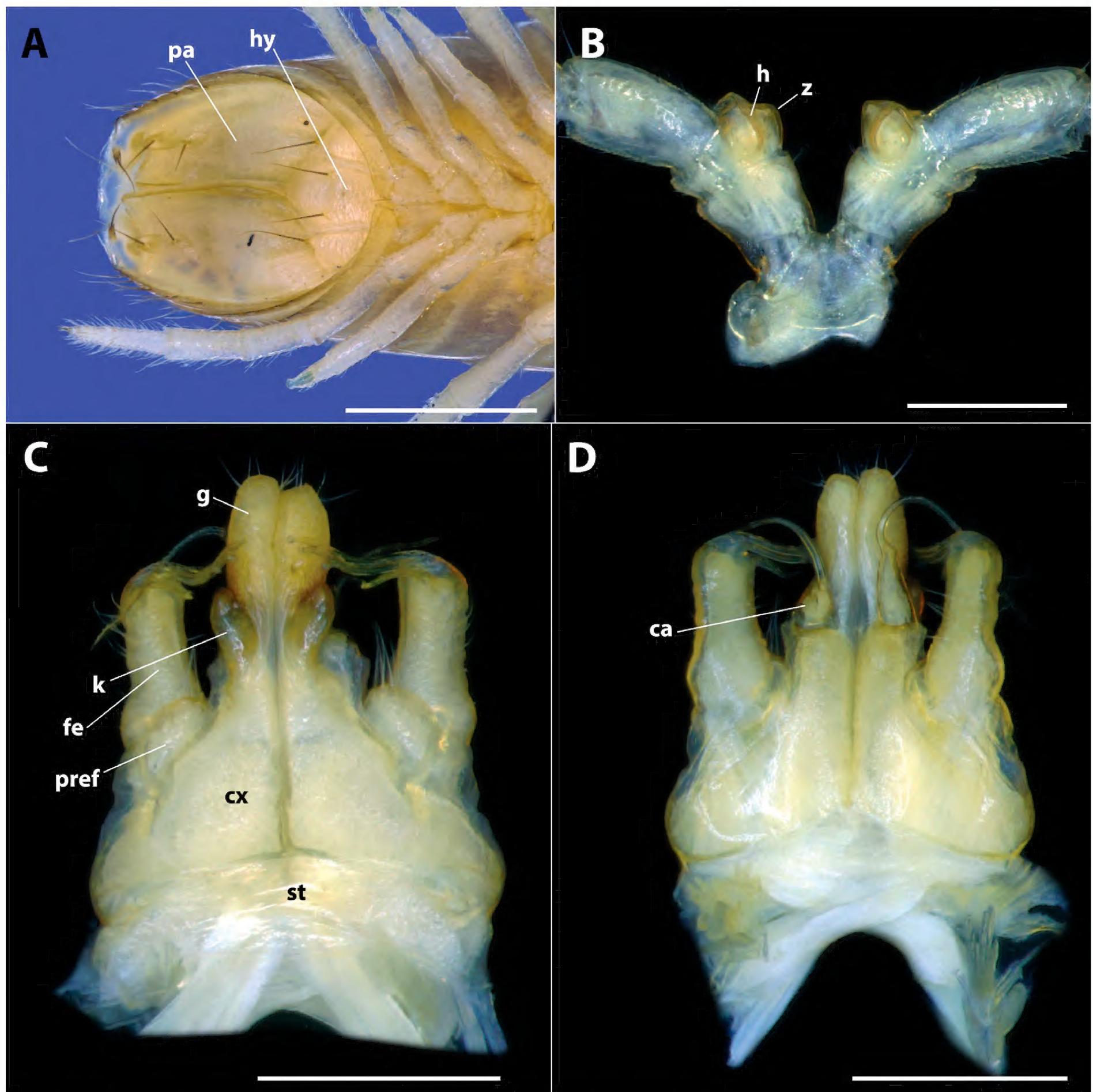


Figure 2. *Sinocallipus deharvengi* Stoev & Enghoff, 2011. ♂, IEBR-Myr 962 **A.** Telson, ventral view; **B.** Leg 9, anterior view; **C, D.** Gonopods, anterior (**C**) and posterior (**D**) views. Abbreviations: pa = paraprocts; hy = hypoproct; h = trochanteral process h; z = trochanteral process z; g = coxal process g; k = coxal process k; st = sternite; cx = coxite; pref = prefemorite; fe = femorite; ca = cannula. Scale bars: 1 mm for (**A**); 0.5 mm for (**B–D**).

and length of gonocoxal process *g* ($2 \times$ as long as process *k* in *S. similis* vs. $3 \times$ as long as process *k* in *S. deharvengi*); from *S. catba* by: body colouration (yellow in *S. similis* vs white in *S. catba*), length of gonocoxal process *g* ($2 \times$ as long as process *k* in *S. similis* vs $1.5 \times$ as long as process *k* in *S. catba*), division of paraproct (paraprocts divided into two almost equal-sized sclerites in *S. similis* vs paraprocts divided into larger ventral and smaller dorsal sclerites in *S. catba*). Pairwise genetic distances for COI between *S. similis* sp. nov., and *S. catba* and *S. deharvengi* are 17.1% and 21.9%, respectively.

Description. Male. Body rings 65–78 PT plus telson (Holotype ♂: 71 PT plus telson). Length 68.4–72.3 mm

(Holotype ♂: 70.5 mm), width of midbody PT 3.2 mm, height of midbody PT 3.4 mm.

Colouration: Body uniformly white-yellowish in alive specimens (Fig. 3D) and yellow in preserved specimens, without particular colouration pattern, metazonites without posterior band. **Head:** Uniformly pale yellow, pilose; cephalic suture visible on vertex (Fig. 4B). **Antennae:** Long, extending beyond the posterior edge of PT 10 when folded backwards; antennomeres, 2 yellow, 3–5 partially brown, 6–7 light yellow (Fig. 4A); length of antennomeres, 1: 0.3 mm, 2: 2.1 mm, 3: 2.5 mm, 4: 1.6 mm, 5: 1.7 mm, 6: 0.9 mm, 7: 0.4 mm; antennomere ratio: $3 > 2 > 5 > 4 > 6 > 7 > 1$; tip of antennomere 7 with four short

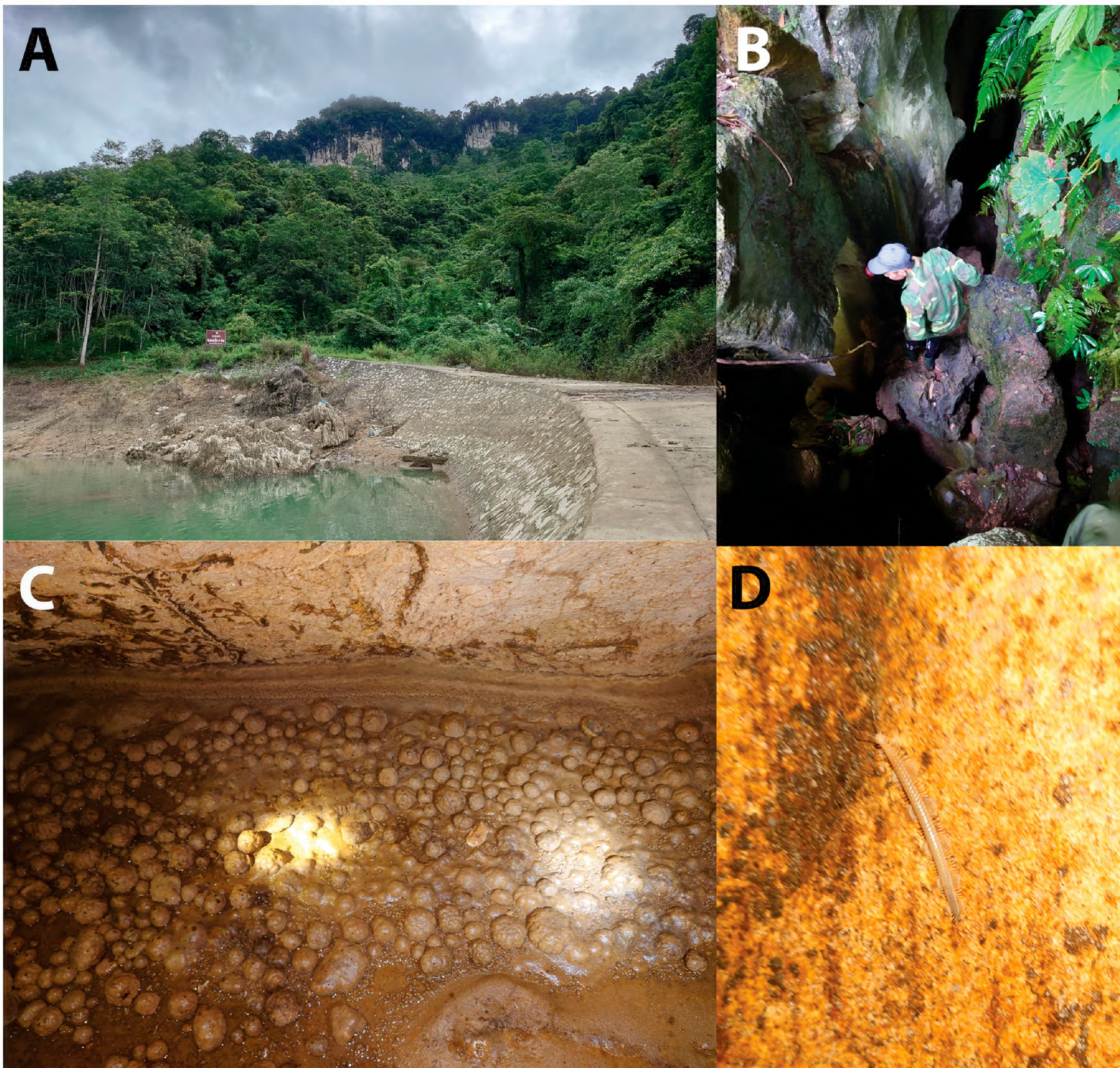


Figure 3. Type locality of *Sinocallipus similis* sp. nov. **A.** Khuoi Lin Cave; **B.** Na Mang Cave; **C.** Ground of Khuoi Lin Cave; **D.** *Sinocallipus similis* sp. nov. habitus.

cones (Fig. 4C). **Eyes:** Black, well-delineated, composed of 32–34 ocelli in 4 rows (Fig. 4D).

Trunk: Width of PT: $1=2=3<4<5<6<7$. PT higher than broad, ratio: 1.06: 1. Dorsal side of collum and PT 2–3 smooth. Crests not well developed, but more obvious dorsally, 5+5 between the ozopores on midbody PT, anterior part of crests broad, abruptly narrowing posteriorly (Figs. 5A, 6A); 8–10 poorly developed crests under the ozopores. Ozopores small, barely visible on PT 5–6, clearly visible, lying on crest 6 in midbody PT, missing on the last 4 PT. **Telson:** Paraprocts divided into two, almost equal-sized dorsal and ventral sclerites; dorsal sclerite surmounted by two macrosetae situated on tiny lobes (Fig. 5C). Spinnerets long and slender, ending with a long seta (Fig. 5B, C). All setae on telson dark brown, contrasting with the yellow background (Fig. 5B).

Legs: All legs yellowish, long and slender, ending with a long claw. Tarsal pads very poorly developed, present on leg pairs 3–12. No particular modifications on coxae of pregonopodal legs. Coxal sacs present on pregonopodal legs (Fig. 5D). Prefemora of legs 4–7 normal.

Leg-pair 9 (Figs 7A, B, 9A): Coxite roundly subtrapezoidal; trochanter expanded medio-ventrad forming a rather elongated process h with a long, pointed tip, and a tiny triangular process z.

Chaetotaxy: All setae broken off. The last two PT each with 9+9 setae.

Gonopods (Fig. 8): Coxite (cx) large and broad; the large coxal process g more than 2x the length of process k, processes g and k apically rounded, not truncated. Prefemorite condensed, short, $0.25 \times$ as long as femorite length. Femoroid (fe) long, but stout, sparsely setose on distally antero-lateral and postero-lateral sides; distal part with three slender,

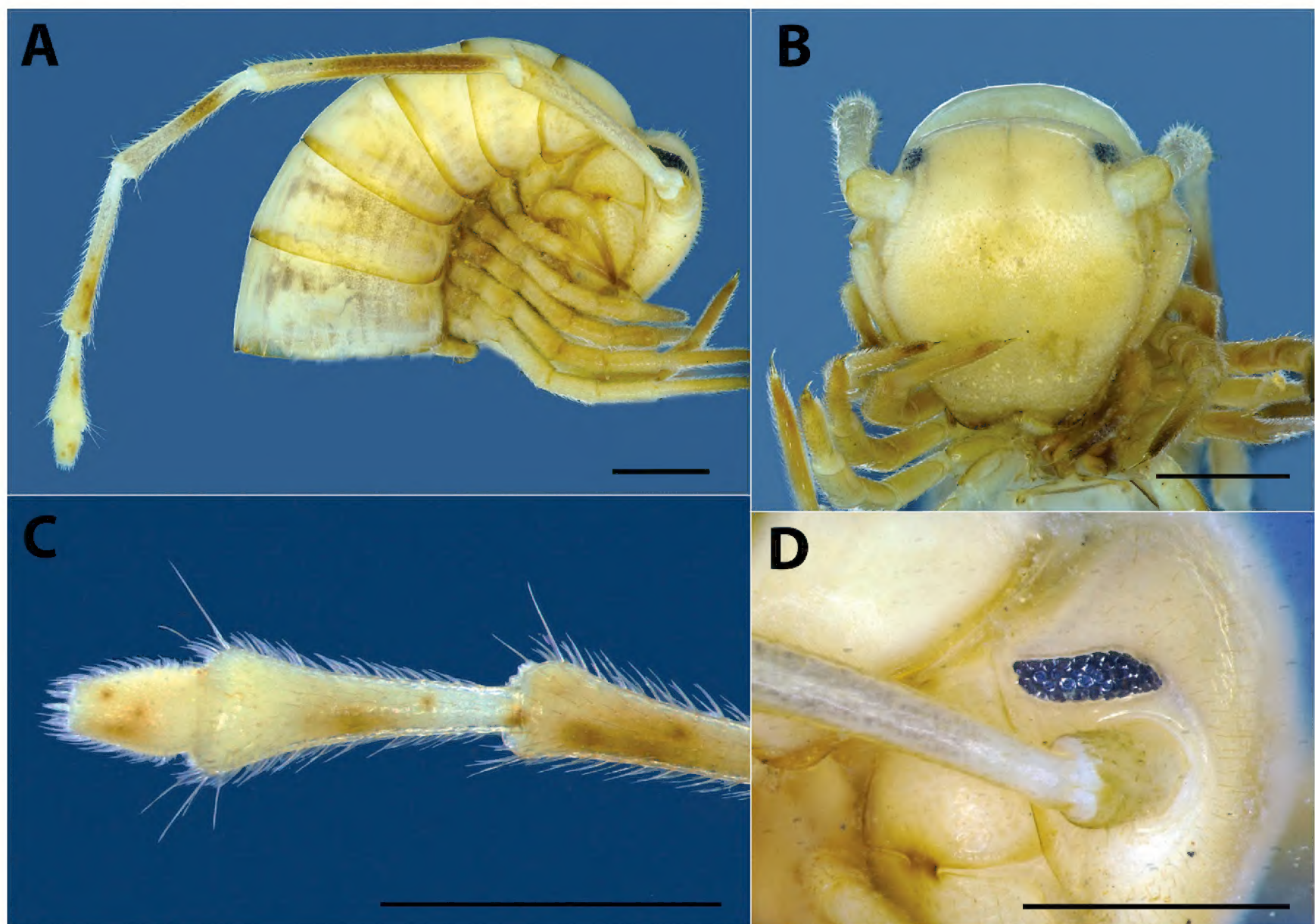


Figure 4. *Sinocallipus similis* sp. nov., holotype ♂ (IEBR-994H). **A.** Anterior segment, lateral view; **B.** Head, anterior view; **C.** Tip of antenna; **D.** Left ocelli (eyes). Scale bars: 1 mm for (A, B); 0.5 mm for (C, D).

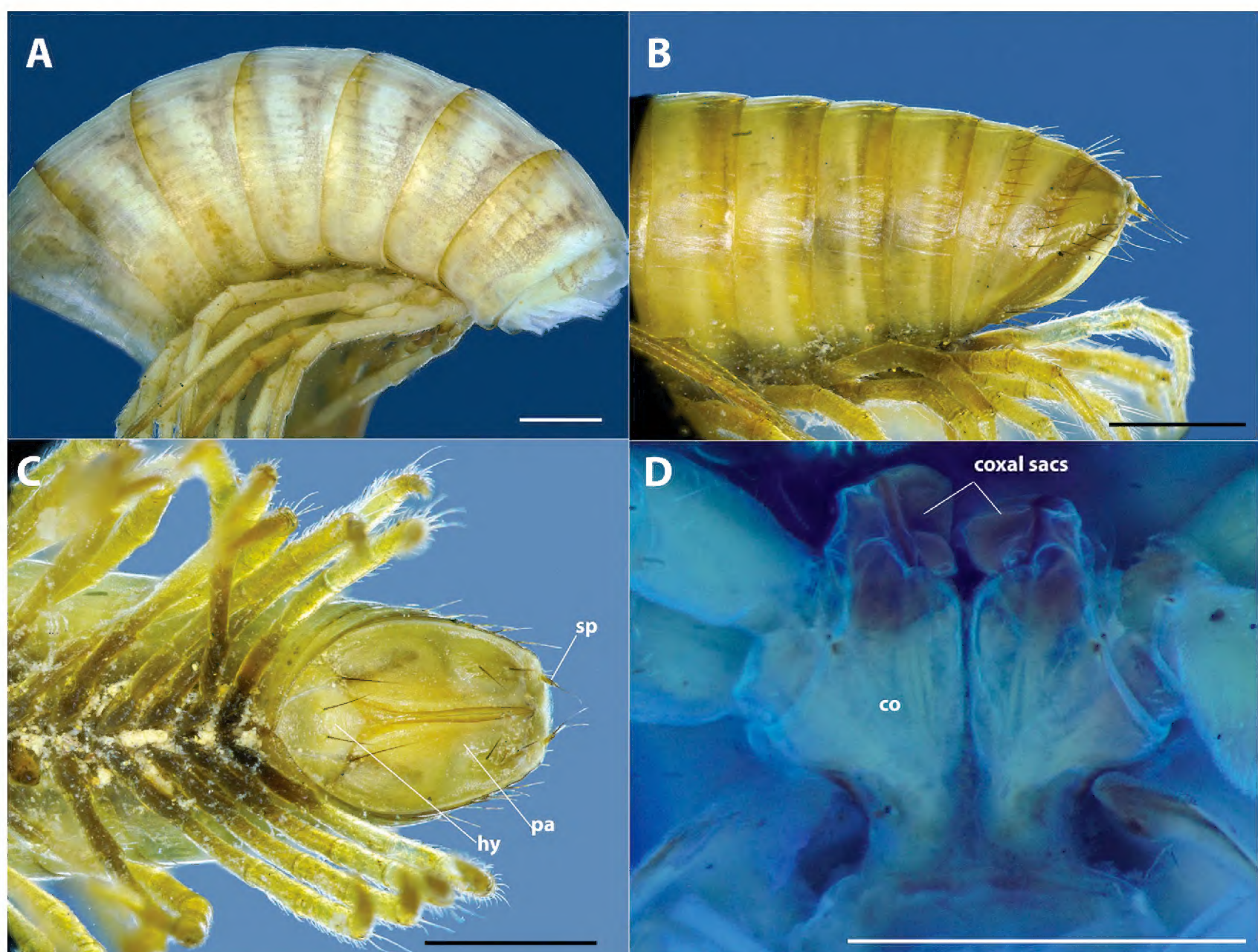


Figure 5. *Sinocallipus similis* sp. nov. Holotype ♂ (IEBR-994H). **A.** Segments 7–13, lateral view; **B, C.** Posterior segments, lateral (B) and ventral (C) views; **D.** Coxal sacs on legs 7, posterior view, under UV light. Abbreviations: pa = paraprocts; hy = hypoproct; cx = coxite; sp = spinneret. Scale bars: 1 mm.

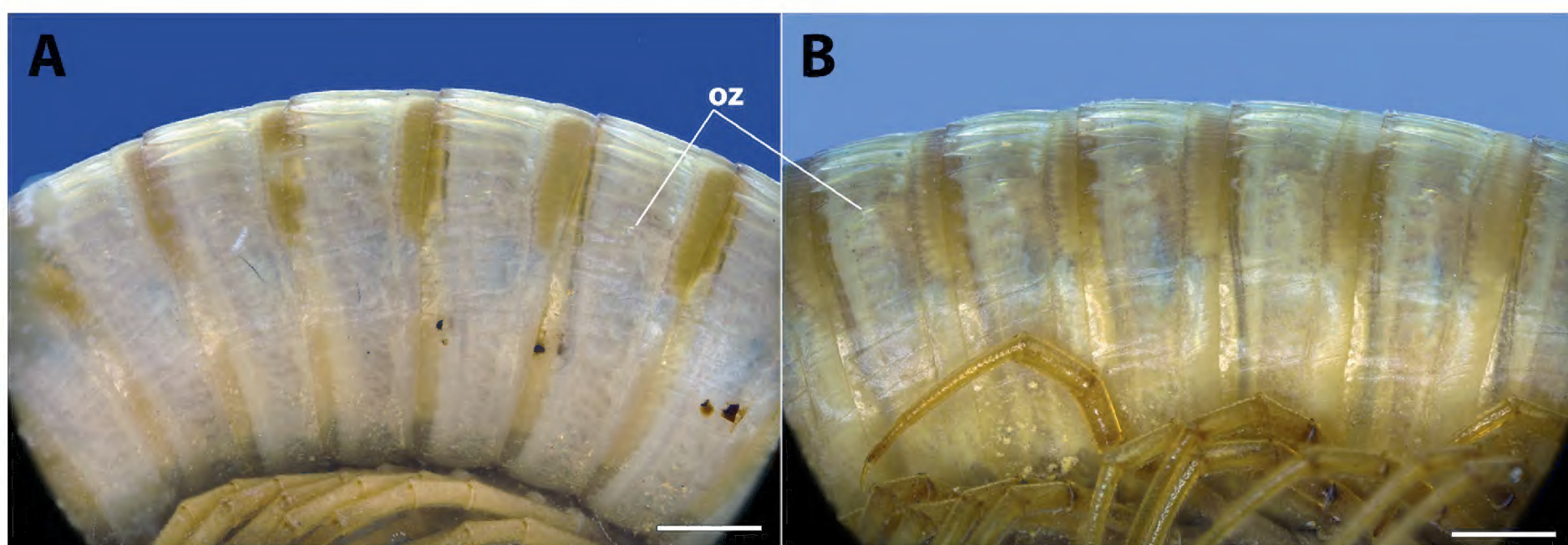


Figure 6. *Sinocallipus similis* sp. nov. Midbody PTs, lateral view. **A.** Holotype ♂ (IEBR-Myr 994H); **B.** Paratype ♀ (IEBR-Myr 994P). Abbreviation: oz = ozopores. Scale bars: 1 mm.

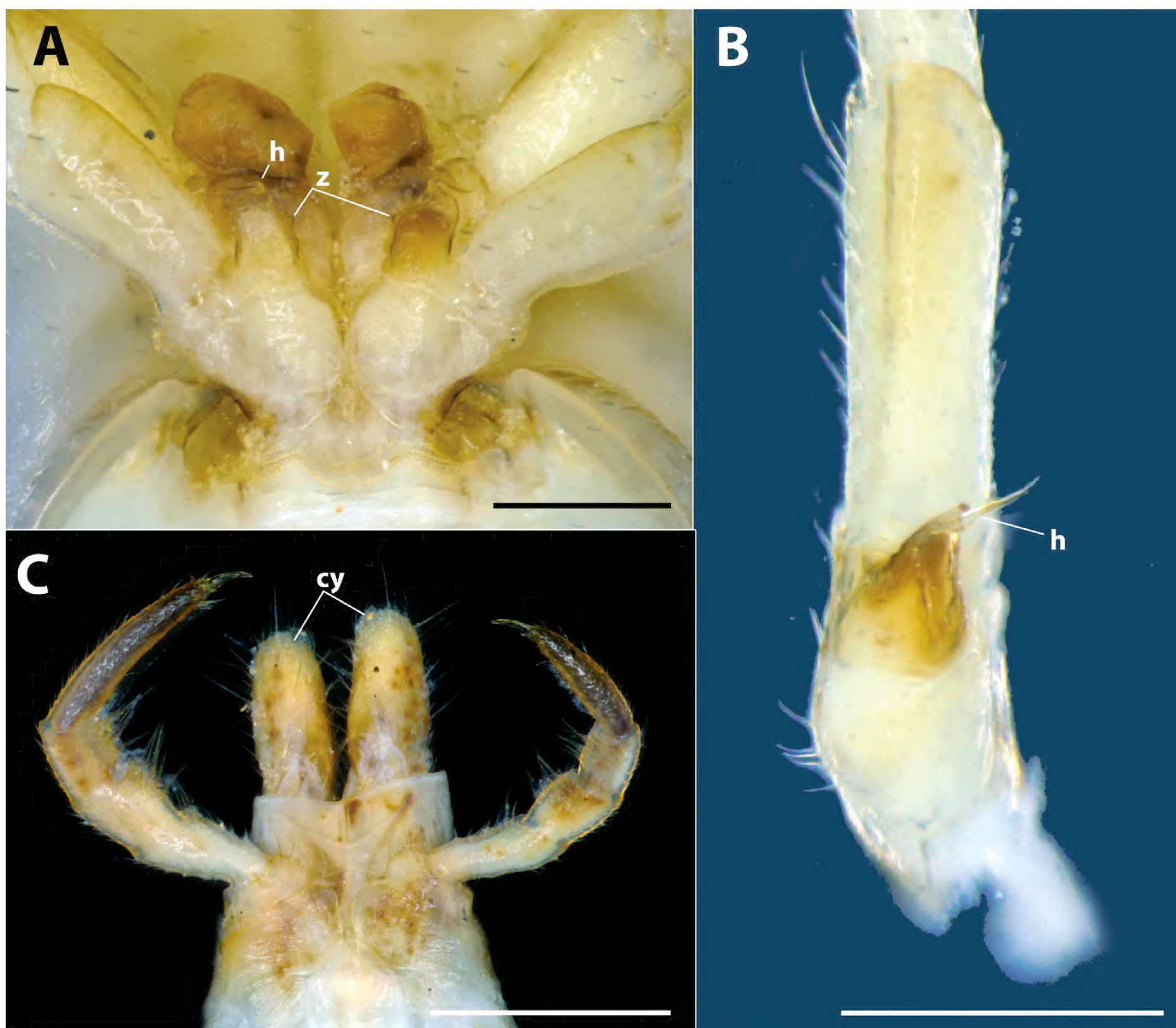


Figure 7. *Sinocallipus similis* sp. nov. Holotype ♂ (IEBR-994H): **A.** Leg 9, anterior view; **B.** Right leg 9, mesal view. Paratype ♀ (IEBR-994P); **C.** Cyphopods, anterior view. Abbreviations: h = trochanteral process h; z = trochanteral process z; cy = cyphopods. Scale bars: 0.5 mm.

acicular (*n*), and one shorter and subfalcate (*m*) terminal projections. Cannula (*ca*): long, slender, filiform.

Female. 75–79 PT + telson; larger than males (length: 72.5–74.8 mm. width of midbody PT: 3.8–4.0 mm; height

of midbody PT: 3.8–4.0 mm, ratio: 1:1), body color darker, lateral sides light brownish; midbody PT crests more developed than in males (Fig. 6B); second leg-pair unmodified; cyphopods cylindrical, highly elevated (Fig. 7C).

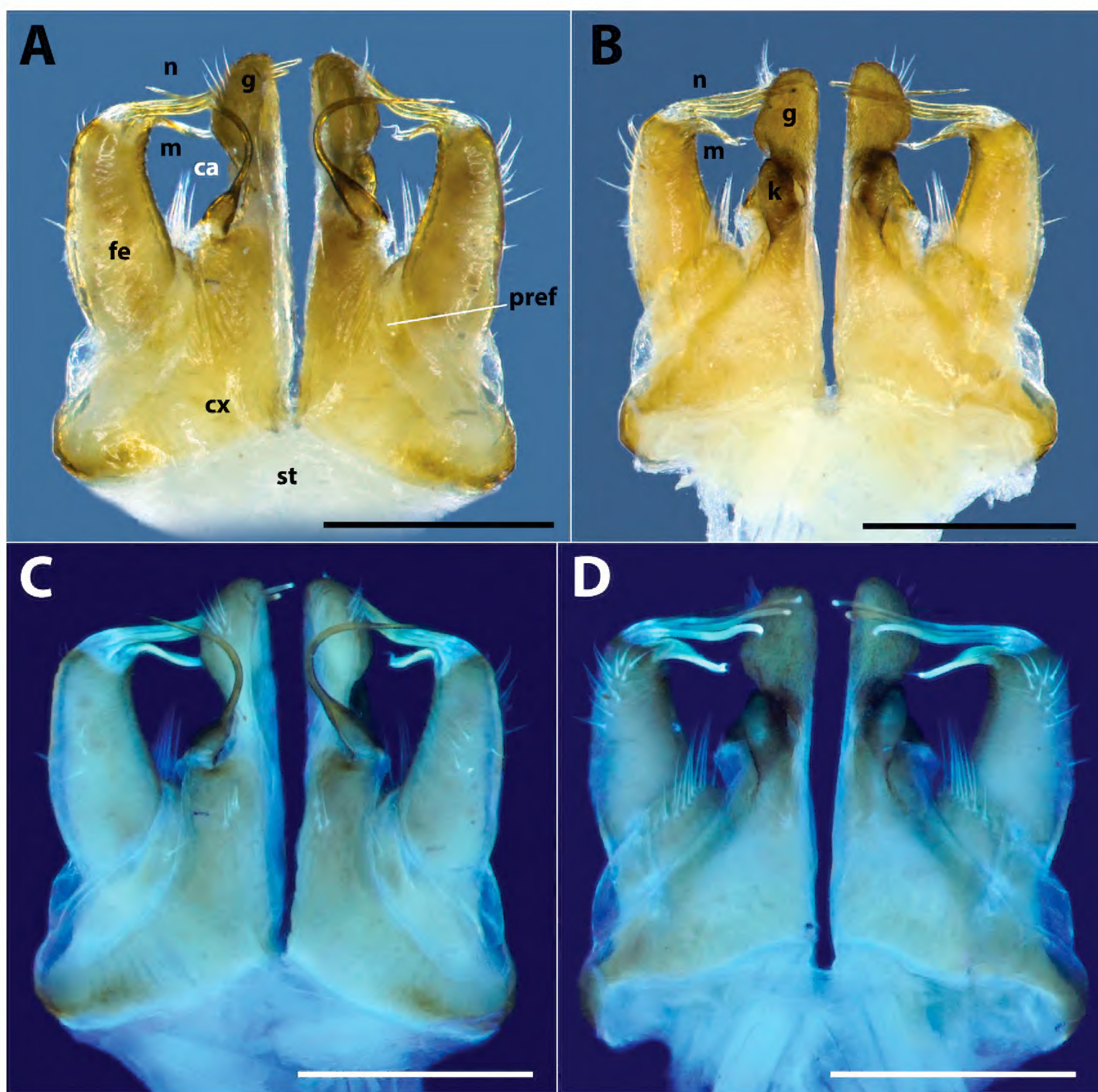


Figure 8. *Sinocallipus similis* sp. nov., holotype ♂ (IEBR-994H), gonopods. **A, B.** Under white light, posterior (**A**) and anterior (**B**) views; **C, D.** Under UV light, posterior (**C**) and anterior (**D**) views. Abbreviations: g = coxal process g; k = coxal process k; m = subfalcate terminal projection; n = acicular processes; st = sternite; cx = coxite; pref = prefemorite; fe = femorite; ca = cannula. Scale bars: 0.5 mm.

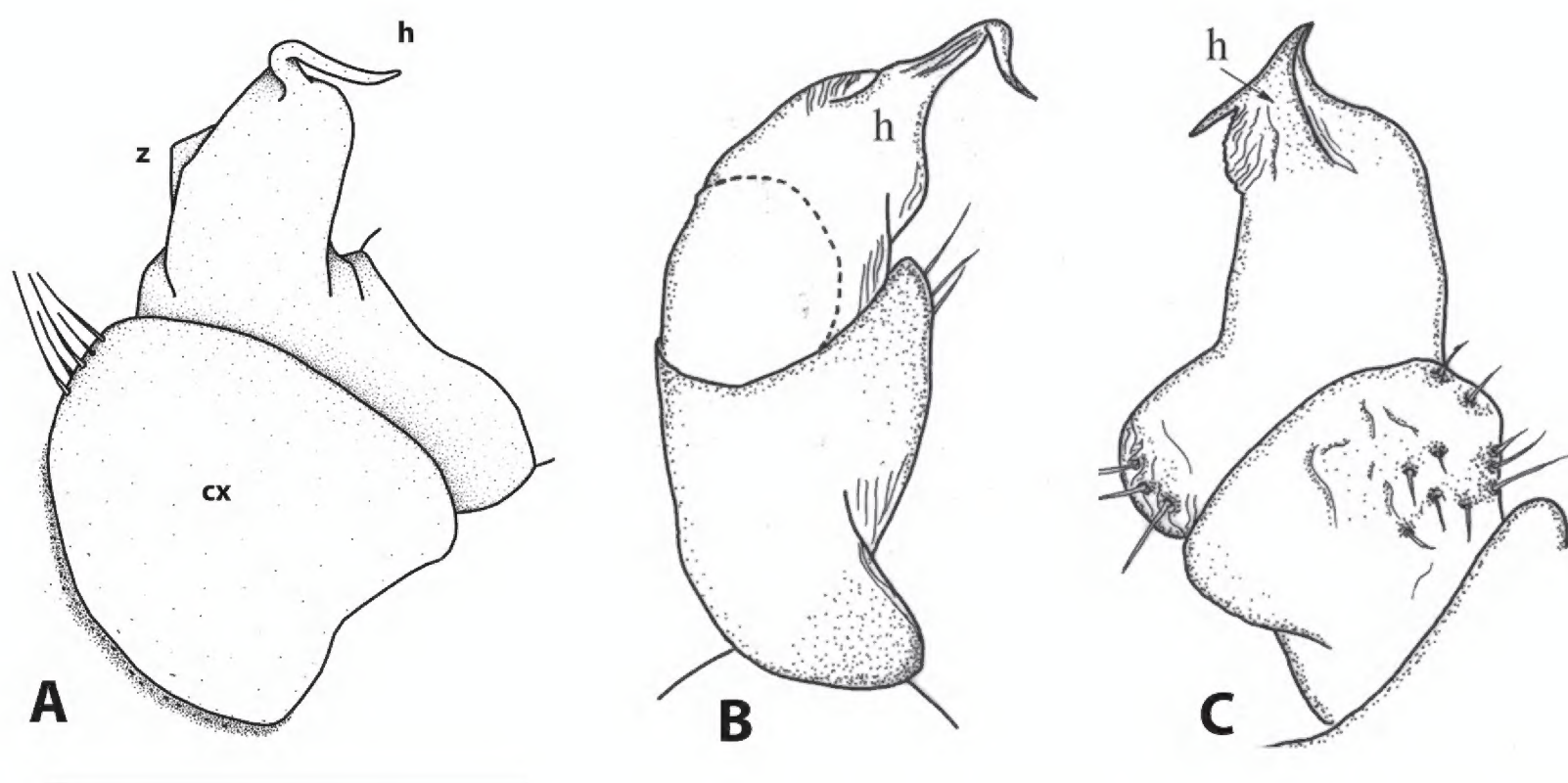


Figure 9. Coxite and trochanteral process of male leg 9. **A.** *Sinocallipus similis* sp. nov.; **B.** *Sinocallipus catba* Stoev & Enghoff, 2011; **C.** *Sinocallipus deharvengi* Stoev & Enghoff, 2011. (**B, C** reproduced from Stoev and Enghoff 2011: figs 26–27). Abbreviations: h = trochanteral process h; z = trochanteral process z; cx = coxite.

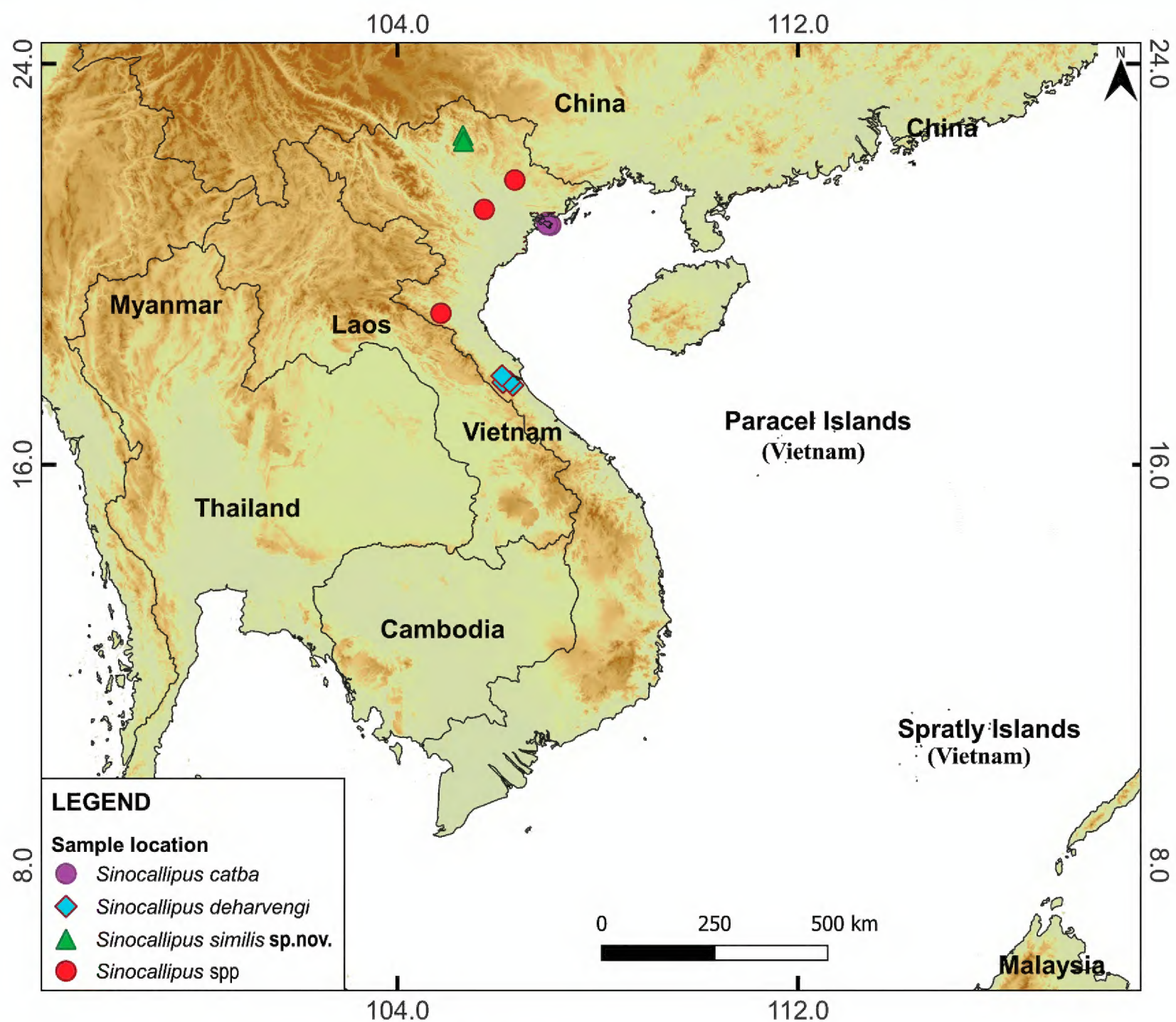


Figure 10. Distribution of *Sinocallipus* Zhang, 1993 in Vietnam.

Sinocallipus sp.

Material examined. VIETNAM • 1♀; Nghe An Province, Pu Mat National Park, unnamed cave, 19.022017°N, 104.86995°E; 79 m a.s.l.; 7 June 2020; Hoang Quang Duy leg.; IEBR-Myr 868.

Remarks. *Sinocallipus* spp. of uncertain taxonomic position have been reported from other regions of Vietnam: Hanoi City (Enghoff et al. 2004), Lang Son Province, Huu Lung Area, Snake Cave, and a cave 97 km north of Hanoi (Stoev and Enghoff 2011).

Molecular analysis

The dataset contains a 560 bp-fragment of COI from 15 samples of 8 callipodidan morphospecies. Outgroup taxa included: six samples of two species of Abacionidae Shelley, 1979 (*Tetracion tennesseensis* Causey, 1959 and *Tetracion jonesi* Hoffman, 1956, from caves in Alabama and Tennessee, USA, respectively) and five samples of two species of Paracortinidae Wang & Zhang, 1993 (*Paracortina* sp. and *Paracortina kyrang* Nguyen et al., 2023, both from northern Vietnam) (Table 1).

Interspecific pairwise genetic distances (K2P) for COI between *S. similis* sp. nov. and its congeners, *S. catba*, *Sinocallipus* sp. from Nghe An Province,

and *S. deharvengi*, are 17.1%, 18.1% and 21.9%, respectively (Table 2). The highest interspecific distance is 24.3% between *S. catba* and *S. deharvengi*. These values indicate significant divergence at the species level within *Sinocallipus*. For comparison, in this study the interspecific genetic distance between the two morphospecies of *Paracortina* analyzed here is 13.8%, while for two species of *Tetracion* it is 9.2%, but 8.8% in Loria et al. (2011). Intraspecific distances are 0.2% for *S. similis* sp. nov. and less than 0.0001% for *P. kyrang*. For *Tetracion* species, the intraspecific distance is 0.2% for *T. jonesi*, and 0.4% for *T. tennesseensis*, but ranged from 0.0–1.4% in *p*-distance (Loria et al. 2011).

The analysis resulted in splitting *Sinocallipus* into two clusters, *S. similis* + *S. catba*, and *S. deharvengi* + *Sinocallipus* sp. from Nghe An Province (Fig. 11). The separation of the two clusters is well supported with relatively high bootstrap support (85%). The distribution of the two clusters corresponds to two different, geographical regions of Vietnam. While the first group is restricted to the karst areas of northeastern Vietnam, which geologically belongs to the southern boundary of the South China block, the two morphospecies of the second group occur in the karst areas of northcentral Vietnam at the eastern edge of the Indochina block (Thanh et al. 2014). However, in each group, the clades between the two species

Table 1. Voucher and GenBank accession numbers for specimens used in this study.

No.	Species	Species code	Locality	GenBank Accession Number	Source
1	<i>Sinocallipus</i> sp.	IEBR-Myr 868	Nghe An Province	PQ685111	This study
2	<i>S. similis</i> sp. nov.	IEBR-Myr 990a	Tuyen Quang Province	PQ685112	
3	<i>S. similis</i> sp. nov.	IEBR-Myr 990b	Tuyen Quang Province	PQ685113	
4	<i>S. catba</i>	IEBR-Myr 542	Hai Phong Province	PQ685114	
5	<i>S. deharvengi</i>	IEBR-Myr 692	Quang Binh Province	PQ685115	
6	<i>Paracortina</i> sp.	IEBR-Myr 1014	Cao Duong commune, Ham Yen District, Tuyen Quang Province	PQ685116	
7	<i>P. kyrang</i> Nguyen, Stoev, Nguyen & Vu, 2023	IEBR-Myr 921	Cao Bang Province	OQ281706	Nguyen et al. (2023)
8	<i>P. kyrang</i> Nguyen, Stoev, Nguyen & Vu, 2023	IEBR-Myr 935	Cao Bang Province	OQ281705	Nguyen et al. (2023)
9	<i>P. kyrang</i> Nguyen, Stoev, Nguyen & Vu, 2023	IEBR-Myr 932	Cao Bang Province	OQ281704	Nguyen et al. (2023)
10	<i>Tetracion tennesseensis</i> Causey, 1959		USA	JN656595	Loria et al. (2011)
11				JN656596	
12				JN656585	
13	<i>T. jonesi</i> Hoffman, 1956		USA	JN656582	Loria et al. (2011)
14				JN656583	
15				JN656579	

Table 2. Pairwise nucleotide difference (Kimura 2-parameter model) over sequence pairs between species. Numbers in bold are divergences between the *Sinocallipus* Zhang, 1993 species.

Species	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
<i>Sinocallipus</i> sp. (1)								
<i>S. similis</i> sp. nov. (2)	0.181	0.002						
<i>S. catba</i> (3)	0.205	0.171						
<i>S. deharvengi</i> (4)	0.217	0.219	0.243					
<i>Paracortina</i> sp. (5)	0.249	0.252	0.262	0.242				
<i>P. kyrang</i> (6)	0.219	0.208	0.229	0.255	0.138	0.000		
<i>Tetracion tennesseensis</i> (7)	0.238	0.236	0.206	0.232	0.193	0.221	0.004	
<i>T. jonesi</i> (8)	0.264	0.230	0.197	0.254	0.205	0.195	0.092	0.002

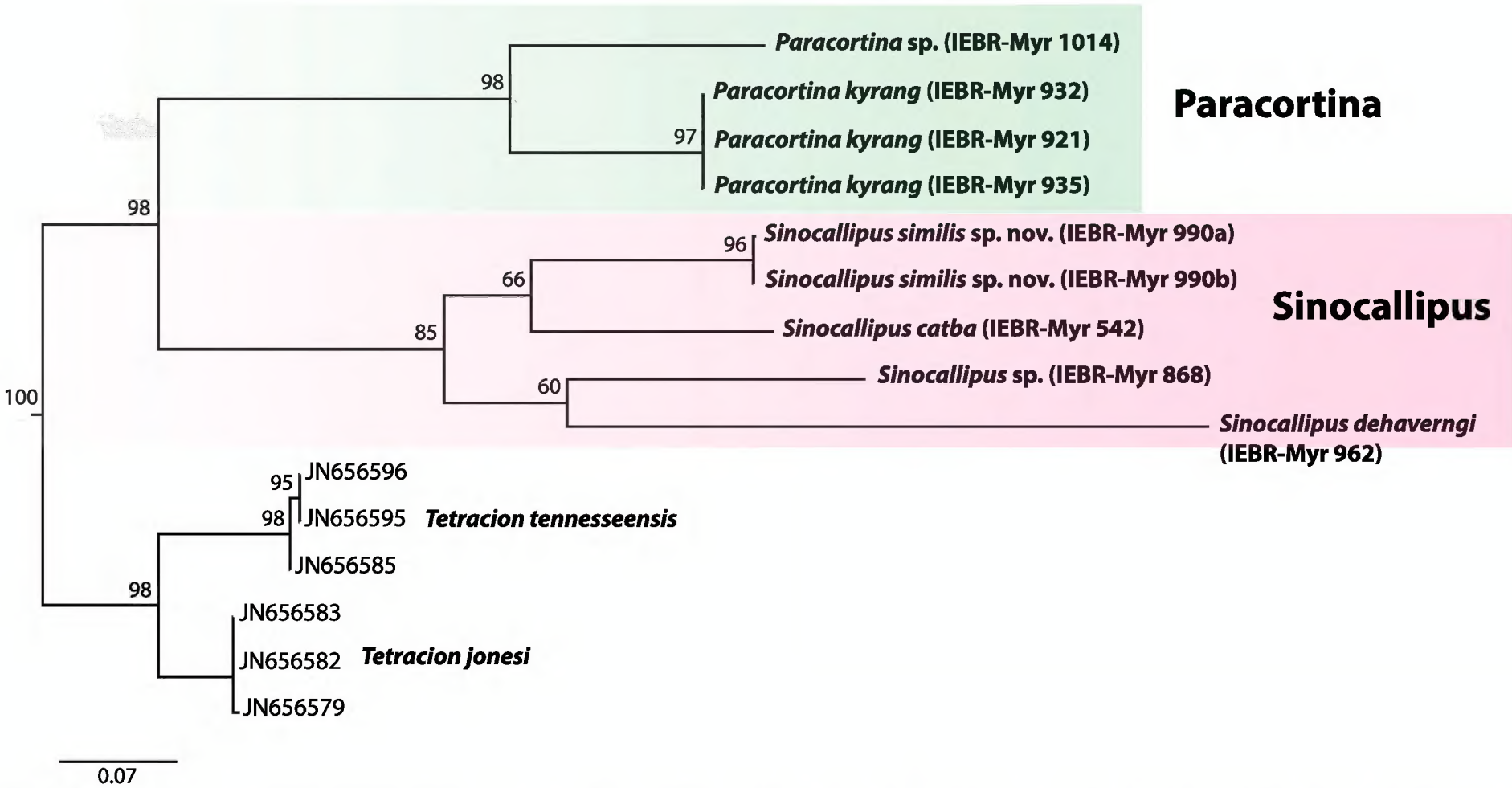


Figure 11. Phylogeny of Vietnamese *Sinocallipus* Zhang, 1993 inferred from a 590 bp fragment of COI using maximum likelihood. Number at nodes are bootstrap values.

were supported by low bootstrap values (66% and 60%, respectively). The low values may underscore unresolved taxonomic relationships within those clusters. However, it requires further studies with more molecular markers and a larger sample size to elucidate the relationships between clusters.

Key to the *Sinocallipus* species in Vietnam

The identification key is made based on gonopodal and somatic characters and complements the key of Stoev and Enghoff (2011). The Vietnamese species are keyed out in Stoev and Enghoff (2011: p. 32, couplet 6 (5)) where the species *Sinocallipus steineri* from Laos, and *S. catba* and *S. deharvengi* are clustered together based on the following characters: “body generally white-yellowish, sometimes mottled gray-brownish; antennae long, extending beyond the posterior edge of PT 7 when folded backwards; eye with less than 40 ocelli; gonopodal femoroid with three slender acicular and one shorter and subfalcate terminal projections”. To this group, we add *S. similis* sp. nov. and present the following revised key to distinguish these four species:

- 1 Length of antennae approx. 5.5 mm; 3+3 crests between ozopores on midbody PT; cave in Luang Prabang Province, Laos..... *S. steineri*
- Length of antennae more than 7 mm; 5+5 crests between ozopores on midbody PT, caves in North and Central Vietnam..... 2
- 2 Gonocoxal process *g* more than 3 × the length of process *k*. Trochanteral process *h* with a comparatively short tip (Fig. 9C); caves in Quang Binh Province, northcentral Vietnam..... *S. deharvengi*
- Gonocoxal process *g* between 1.5–2 × the length of process *k*. Trochanteral process *h* with a long tip (Fig. 9A, B); caves in northern Vietnam 3
- 3 Body white, maximal length up to 50 mm; gonocoxal process *g* long, ca. 1.5 × the length of process *k*; paraprocts divided into larger ventral and smaller dorsal sclerites; caves on Cat Ba Island, Vietnam *S. catba*
- Body yellow, maximal length extends 70 mm (72.3 mm); gonocoxal process *g* about 2 × the length of process *k*; paraprocts almost equally subdivided; caves in Tuyen Quang Province, northern Vietnam..... *S. similis* sp. nov.

Discussion

Stoev and Enghoff (2011) discussed the role of gonopod morphology in species differentiation. They noted that gonopod morphology among species of *Sinocallipus* has little variation, making it unsuitable for reliable species identification. This has also been demonstrated here in the case of the three Vietnamese species: *S. similis* sp. nov., *S. catba*, and *S. deharvengi*. All species have extremely similar gonopod conformation, for example: coxal processes *g* and *k* swollen, apically rounded, femoroid with three slender, acicular, and one short and subfalcate terminal projections, cannula long and slender. The only difference between the three species is the relative length of coxal processes *g* and *k*, and the trochanteral process *h* of leg 9. Although these *Sinocallipus* species may not differ much in gonopod morphology, they can be distinguished by molecular data. The genetic divergence (17.1–24.3% in COI) supports the separation of these three species. In addition, our phylogenetic tree also supports the separation of the four *Sinocallipus* species, including an *incertae sedis* one.

The genus *Sinocallipus* is currently found in southern China, north-central Vietnam, Laos, and extends southward to central Thailand (Stoev and Enghoff 2011). Most species, except for *S. thai*, which was collected under a rock at the base of a limestone hill near Sriwilai Cave Temple in Thailand, have been discovered in caves (Stoev et al. 2007; Stoev and Enghoff 2011). Similarly, *S. jaegeri* occurs both in caves and under stones. Specimens of uncertain species identity have also been found outside caves in Vietnam and Laos (Enghoff et al. 2004; Shear et al. 2003). Overall, members of the genus are generally confined to caves and karst environments.

Vietnam is known for its rich karst regions in northern and north-central areas. Several cave-dwelling species

have been reported, including spirostreptidan, *Hyleoglomeris* Verhoeff, 1910 and polydesmidan millipedes (Golovatch et al. 2007, 2009a, 2009b; Golovatch 2019; Kuroda et al. 2022; Nguyen et al. 2024). The number of described species is still far from representing the true diversity of all troglobiotic species in Vietnam. Additionally, more cave-dwelling glyphiulids and haplodesmids are deposited in the Myriapod collection of Institute of Ecology and Biological Resources (Hanoi), awaiting description. It is expected that new species will be discovered if more intensive surveys are conducted in Vietnam’s karst regions.

Acknowledgements

The work is supported by the Vietnam Academy of Science and Technology under the project NCXS01.04/23-25 “Developing the first-class research team on the discovery of diversity and application potential of hymenopterans, myriapods and soil nematodes in the limestone mountains of northeastern Vietnam”. For this study, Pavel Stoev utilized the scientific equipment and laboratories of the National Museum of Natural History at the Bulgarian Academy of Sciences, which were acquired or upgraded through the DiSSCo-BG project (Distributed System of Scientific Collections-Bulgaria), a project funded by the National Roadmap for Research Infrastructures, Ministry of Education and Science of the Republic of Bulgaria. Ms Pham Mai Phuong (Vietnam-Russian Tropical Research Center, Hanoi) was thanked for kindly preparing the distribution map. Three reviewers, Stephanie Loria (USA), Natdanai Likhitrakarn (Thailand), and Rodrigo Bouzan (Brazil) were acknowledged for their constructive comments to improve the manuscript.

References

- Causey NB (1959) Some cavernicolous millipeds from the Cumberland Plateau. *Journal of the Tennessee Academy of Science* 34: 229–237.
- Enghoff H, Golovatch SI, Nguyen DA (2004) A review of the millipede fauna of Vietnam. *Arthropoda Selecta* 13: 25–43.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294–299.
- Golovatch SI (2019) On several new or poorly-known Oriental Paradoxosomatidae (Diplopoda: Polydesmida), XXVII. *Arthropoda Selecta* 28: 459–478. <https://doi.org/10.15298/arthscl.28.4.01>
- Golovatch SI, Geoffroy J-J, Mauriès J-P, Van den Spiegel D (2007) Review of the millipede genus *Glyphiulus* Gervais, 1847, with descriptions of new species from Southeast Asia (Diplopoda, Spirostreptida, Cambalopsidae). Part 2: the *javanicus*-group. *Zoosystema* 29: 417–456.
- Golovatch SI, Geoffroy J-J, Mauriès J-P, VandenSpiegel D (2009a) Review of the millipede genus *Eutrichodesmus* Silvestri, 1910 (Diplopoda, Polydesmida, Haplodesmidae), with descriptions of new species. *ZooKeys* 12: 1–46. <https://doi.org/10.3897/zookeys.12.167>
- Golovatch SI, Geoffroy J-J, Mauriès J-P, Van den Spiegel D (2009b) Review of the millipede genus *Plusioglyphiulus* Silvestri, 1923, with descriptions of new species from Southeast Asia (Diplopoda, Spirostreptida, Cambalopsidae). *Zoosystema* 31: 71–116. <https://doi.org/10.5252/z2009n1a5>
- Hoffman RL (1956) New genera and species of cavernicolous diplopods from Alabama. *Geological survey of Alabama, Museum Paper* 35: 5–13.
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Kuroda M, Eguchi K, Oguri E, Nguyen AD (2022) Two new cave *Hyleoglomeris* species (Glomerida, Glomeridae) from northern Vietnam. *ZooKeys* 1108: 161–174. <https://doi.org/10.3897/zookeys.1108.85423>
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>
- Loria S, Zigler K, Lewis J (2011) Molecular phylogeography of the troglomorphic millipede *Tetracion* Hoffman, 1956 (Diplopoda, Callipodida, Abacionidae). *International Journal of Myriapodology* 5: 35–48. <https://doi.org/10.3897/ijm.5.1891>
- Nguyen AD, Korsós Z, Jang KH, Hwang UW (2017) A revision and phylogenetic analysis of the millipede genus *Oxidus* Cook, 1911 (Polydesmida: Paradoxosomatidae). *European Journal of Taxonomy* 293: 1–22. <https://doi.org/10.5852/ejt.2017.293>
- Nguyen AD, Stoev P, Nguyen LTP, Vu TT (2023) A new species of *Paracortina* from a Vietnamese cave, with remarkable secondary sexual characters in males (Callipodida, Paracortinidae). *ZooKeys* 1149: 181–195. <https://doi.org/10.3897/zookeys.1149.99651>
- NguyenAD, Vu TTT, Eguchi K (2024) The millipede family Polydesmidae Leach, 1816 (Diplopoda, Polydesmida) from Vietnam, with a description of a new cavernicolous species. *ZooKeys* 1190: 259–280. <https://doi.org/10.3897/zookeys.1190.114958>
- Shear W (2000) A new genus and species of callipodidan millipede from Vietnam (Callipodida, Schizopetalidae). *Myriapodologica* 6: 95–100.
- Shear W, Shelley R, Heatwole H (2003) Occurrence of the millipede *Sinocallipus simplipodicus* Zhang, 1993 in Laos, with reviews of the Southeast Asian and global callipodidan faunas, and remarks on the phylogenetic position of the order (Callipodida: Sinocallipodidea: Sinocallipodidae). *Zootaxa* 365: 1–20. <https://doi.org/10.11646/zootaxa.365.1.1>
- Shelley RM (1979) A revision of the millipede genus *Delophon* with the proposal of two new tribes in the subfamily Abacioninae (Callipodida: Caspiopetalidae). *Proceedings of the Biological Society of Washington* 92: 533–550.
- Stoev P, Enghoff H (2011) A review of the millipede genus *Sinocallipus* Zhang, 1993 (Diplopoda: Callipodida: Sinocallipodidae), with notes on gonopods monotony vs. peripheral diversity in millipedes. *ZooKeys* 90: 13–34. <https://doi.org/10.3897/zookeys.90.1291>
- Stoev P, Enghoff H, Panha S, Fuangarworn M (2007) A second species in the millipede suborder Sinocallipodidea Shear, 2000 (Diplopoda: Callipodida). *Zootaxa* 1450: 63–68. <https://doi.org/10.11646/zootaxa.1450.1.6>
- Thanh NX, Hai TT, Hoang N, Lan VQ, Kwon S, Itaya T, Santosh M (2014) Backarc mafic-ultramafic magmatism in Northeastern Vietnam and its regional tectonic significance. *Journal of Asian Earth Sciences* 90: 45–60. <https://doi.org/10.1016/j.jseaes.2014.04.001>
- Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ (2016) IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44: W232–W235. <https://doi.org/10.1093/nar/gkw256>
- Wang D, Zhang C (1993) A new family of millipedes (Diplopoda: Callipodida) from southwestern China. *Peking National History Museum Memoirs* 53: 375–390.
- Zhang C (1993) Diplopoda from Yunnan caves. II. Contribution to the study of a new cavernous taxon of the nematophoran millipedes (Diplopoda: Coelocheta: Callipodida). In: *Proceedings of the XI International Congress of Speleology*. Beijing, China, 128–130.
- Zhang Z, Schwartz S, Wagner L, Miller W (2000) A Greedy Algorithm for Aligning DNA Sequences. *Journal of Computational Biology* 7: 203–214. <https://doi.org/10.1089/10665270050081478>